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A taste of youth: Seasonal changes in the diet of immature white sharks in eastern Australia

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White sharks (*Carcharodon carcharias*) play a crucial ecological role, shaping ecosystems through direct predation and risk effects. On the east coast of Australia, immature white sharks are broadly distributed, inhabiting a wide range of habitats and ecosystems from temperate Tasmania to tropical North Queensland. Using stable isotopes and fatty acids of muscle and plasma, we examined the diet and habitat use of 136 immature white sharks (152–388 cm total length) captured on SMART drumlines on the East Australian coast. This facilitated the temporal assessment of white shark trophic ecology from a few weeks to approximately a year. Biochemistry of muscle samples showed that white sharks predominantly feed on low trophic level prey from coastal environments. A seasonal shift in diet was evident, with the increasing proportions of essential fatty acids in muscle tissues during spring and summer suggesting a greater consumption of high-nutrition preys during those months compared to autumn and winter. By combining stable isotope and fatty acid analysis, we gain a comprehensive understanding of immature white shark diet in eastern Australia. Our results confirm that white sharks are generalist predators that exhibit seasonal shifts in their diet. Their high use of coastal habitats reinforces the importance of these areas for foraging, which is crucial for growth and development during this critical life stage.

KEYWORDS

Stable isotopes, fatty acids, trophic ecology, biomarkers, marine predator

1 Introduction

Highly mobile marine predators play crucial ecological roles by connecting spatially separated food webs and shaping ecosystems through diet and habitat use (Heithaus and Dill, 2002; Heithaus et al., 2010; Williams et al., 2018). In marine ecosystems, diet and energetic requirements impact an individual's ability to execute intrinsic biological processes necessary for migration, reproduction, and survival (de Sousa Rangel et al., 2021a). Such energetic demands vary throughout an animal's life stages, influencing trophic role, foraging preferences and capabilities throughout ontogeny (Kohl et al., 2015; Chaguaceda et al., 2020). The trophic ecology of many marine predators, such as sharks, is well documented (Hussey et al., 2015) but often focuses on large mature individuals (Heithaus and Dill, 2002; Huveneers et al., 2018). Understanding the diet of marine predators across all life stages is essential, as resource availability and feeding preferences influence shark habitat selection, particularly in early life stages (Krausman, 1999; Heithaus, 2007).

Insight into the diet of marine predators is often limited to brief snapshots of recently consumed prey from direct observations of predation, stomach content analysis, or, more recently, genetic analyses of faecal material (Munroe et al., 2018; Clark et al., 2023). However, relying solely on snapshots of recently ingested meals may lead to inaccurate assessments of diet and foraging patterns because most recent meals might not represent a species' diet comprehensively. Alternative indirect measures, e.g., biochemical tracers, provide valuable information over broader and more ecologically relevant spatial and temporal scales and are used to quantify and estimate resource use over weeks to years (Hussey et al., 2011; Pethybridge et al., 2014; Raoult et al., 2019; Meyer et al., 2021). Based on the principle 'you are what you eat', elements and compounds within prey items are assimilated into consumer tissue with minimal or predictable modification and can be traced upwards from the base of the food chain (Iverson et al., 2004; Budge et al., 2006; Munroe et al., 2018). Biochemical tracers can reveal diet, feeding patterns, and habitat use (Carlisle et al., 2021; Meyer et al., 2021), with short-term information (~30 days) gained from metabolically active tissues like plasma and liver (Hussey et al., 2012a), while less active tissue like muscle provides longer-term (months to years) information (Kim et al., 2012a).

Stable isotopes of carbon and nitrogen are widely used biochemical tracers owing to their ability to quantify basal carbon sources and trophic levels, respectively. This enables stable isotope analyses to elucidate a predator's dietary composition and specialisation, ontogenetic changes, variations in habitat use, and ecological dynamics within a community (Peterson and Fry, 1987; Carlisle et al., 2012; Pethybridge et al., 2018). Additionally, the use of lipids and fatty acids can be used to infer the diet, habitat use, nutritional condition, and physiology of elasmobranchs (Pethybridge et al., 2011; Gallagher et al., 2017; Meyer et al., 2017; Munroe et al., 2018). Fatty acids are critical for physiological functions, including growth, development, reproduction, and cellular maintenance (Sargent et al., 1995; Tocher, 2003). However, elasmobranchs have limited capacity for lipid oxidation in extrahepatic tissues (Zammit and Newsholme, 1979; Ballantyne, 1997), and essential fatty acids, including arachidonic (ARA), eicosapentaenoic (EPA), and docosahexaenoic (DHA), cannot be synthesised *de novo* (Sargent et al., 1995). Therefore, fatty acids must be obtained through diet, and many remain relatively unchanged, enabling their use as dietary biomarkers (Dalsgaard et al., 2003; Iverson, 2009). Fatty acid incorporation in tissues is substantially quicker than stable isotopes, with shifts in consumer nutrition reflected in muscle and blood fatty acid profiles within weeks rather than months or years (Beckmann et al., 2013). Subsequently, fatty acids are useful biomarkers, providing a fine-scale assessment of spatiotemporal changes in diet and nutrition (Rohner et al., 2013; Alderete-Macal et al., 2020; Meyer et al., 2021).

On the east coast of Australia, juvenile and sub-adult white sharks (hereafter referred to as immature white sharks) are broadly distributed, inhabiting a wide range of coastal habitats and ecosystems from temperate Tasmania and Bass Strait (Victoria) in summer to tropical regions as far north as the southern Great Barrier Reef (Queensland) in winter (Bruce and Bradford, 2012; Bruce et al., 2019). Telemetry studies reveal that immature white sharks undertake seasonal north-south migrations, spending increased time in southern Queensland and northern New South Wales from September, before moving to southern New South Wales and Victorian waters in March (Spaet et al., 2020b). Similar patterns of seasonal movement have been reported in the North Atlantic (Skomal et al., 2017; Franks et al., 2021), South Africa (Towner et al., 2013), and the north-east Pacific (Weng et al., 2007). Although highly migratory, immature white sharks spend more time in so-called 'nursery' areas between Lake Macquarie (33.3°S) and South West Rocks (30.8°S), returning in consecutive years (Bruce et al., 2019).

Previous studies using stomach contents and fatty acids, and more recently environmental DNA (eDNA) metabarcoding, indicate the diet of these white sharks comprises primarily of finned fishes, benthic batoids, squid and marine mammals (Pethybridge et al., 2014; Grainger et al., 2020; Clark et al., 2023). However, inconsistencies between these studies are evident. Stomach contents evaluated by Grainger et al. (2020) found Australian salmon (*Arripis trutta*) had high occurrence, compared to sea mullet (*Mugil cephalus*) using eDNA metabarcoding by Clark et al. (2023). These discrepancies highlight the variability in white shark diet likely attributed to spatial and temporal differences within the sampling regime. Earlier studies (Pethybridge et al., 2014; Grainger et al., 2020) relied on opportunistic sampling from fisheries bycatch or shark meshing programs, with sample sizes of 21 and 52, respectively, and limited samples collected during winter, when white shark presence is high Spaet et al. (2020b). Although the novel method of environmental DNA described by Clark et al. (2023) provides a higher taxonomic resolution than biochemical tracers, a lack of reference sequence data may result in underrepresentation, while sample contamination from the surrounding environment may lead to false positives (Beng and Corlett, 2020). These studies have provided valuable insight into the diet of east coast white sharks, but temporal changes in the diet of immature white sharks in this region are yet to be described. Seasonal fluctuations in prey abundance are persistent on the east coast of Australia (Brodie et al., 2017), and the dynamic

oceanographic conditions influence prey distribution (Hobday et al., 2011).

The East Australian Current (EAC) is a western boundary current that flows from the Coral Sea, southward into New South Wales (Ridgway and Godfrey, 1997). Seasonal north-south expansion and contraction of the East Australian Current, in addition to high mesoscale kinetic energy, facilitates the distribution of chlorophyll *a* along the coastline (Everett et al., 2014; Liu et al., 2022). In northern areas at latitudes between 28.5°S and 31°S, the shelf narrows, leading to upwelling of nutrient-rich water and increases in primary productivity (Everett et al., 2014). The EAC has the strongest influence on shelf waters during late spring and summer, affecting the abundance and distribution of large marine predators along the east coast (Young et al., 2010; Hobday et al., 2011; Lee et al., 2021). Therefore, predators may require plasticity in habitat use and foraging patterns to meet energy and nutritional requirements in such fluctuating environments. Adaptation and responses to ephemeral prey availability remain species-specific (Terraube et al., 2011; Munroe et al., 2014), yet vital, as predators with the capability to adapt will be able to cope better with the forecasted impacts on environments and preys in the Anthropocene.

In the present study, we detail the trophic ecology of immature white sharks along Australia's east coast across temporal scales by using biochemical tracers and tissues reflecting diet from a few weeks up to a year. Specifically, we calculate annual and seasonal diet composition and infer foraging habitat from nitrogen and carbon stable isotopes. Fatty acid signatures of muscle and plasma were also used to investigate shifts in diet and trophic pathways. Together, these methods provide an integrated understanding of immature white shark diet and habitat use within a highly dynamic coastal ecosystem. Based on the documented seasonal movements of immature white sharks through acoustic and satellite telemetry and seasonal changes in prey distribution in this region, we hypothesised stable isotope and fatty acid values to reflect a seasonal shift in diet and nutritional condition.

2 Materials and methods

To quantify the diet and examine the foraging habitat of white sharks, 102 muscle and 39 blood samples (136 individuals; 8 sharks had both muscle and blood collected) were collected from sharks caught between Ballina (28.811° S 153.610° E) and Forster (32.180° S 152.511° E), New South Wales, Australia, from 9 August 2016 to 3 February 2022 (Figure 1). We captured sharks using SMART (Shark-Management-Alert-in-Real-Time) drumlines deployed in coastal waters as part of the New South Wales Department of Primary Industries (NSW DPI) Shark Management Program (see Lipscombe et al., 2023 and Tate et al., 2021 for gear configuration and deployment). Previous studies have shown that the time spent on the line using this capture method does not impact plasma fatty acids, enabling the confident use of plasma fatty acids to examine diet and habitat use (Gallagher et al., 2019; Tate et al., 2019). We recorded sex and size (total length [TL] to the nearest cm). Sharks ranged from 152–388 cm TL, including six young-of-the-year < 175 cm TL, 119 juveniles 176–300 cm TL, and 11 sub-adults 300–388 cm TL (Bruce and Bradford, 2012).

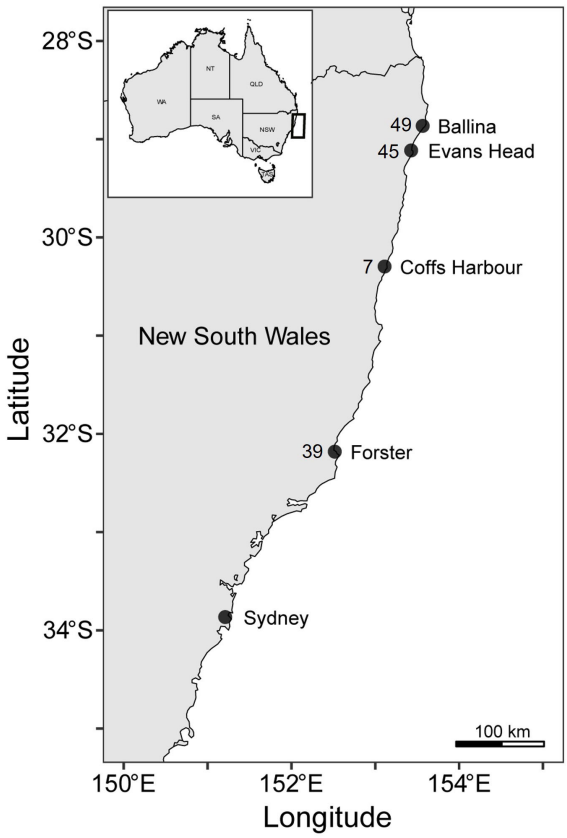

FIGURE 1
Sampling location and number of samples collected from white sharks in New South Wales, Australia.

2.1 Sample collection and preparation

We sampled the epaxial muscle adjacent to the dorsal fin using a hand-held 1 cm diameter stainless steel biopsy and stored on ice before transferring to a -18°C freezer. We collected blood via caudal venipuncture and placed into a lithium-heparin plasma separator vial (BD Vacutainer), centrifuged at 1534 ×*g* for 3 min and pipetted the plasma into 2 mL vials. We stored the plasma samples temporarily at -18°C in the field and later transferred them to a freezer at -80°C.

Lipids were extracted from samples for subsequent fatty acid analysis to ensure lipid content did not bias carbon isotopes (Newsome et al., 2010; Carlisle et al., 2017). We lyophilised muscle (0.1 ± SD 0.01 g) and plasma (1.2 ± SD 0.78 g) for 48 h (Alpha LD14 plus freeze dryer) and homogenised the samples in a TissueLyser LT (Qiagen) for 2 min at 50 Hz to facilitate optimal lipid extraction. Using a modified Bligh and Dyer (1959) method, lipids were extracted from the lyophilised tissue using 3.8 mL of dichloromethane:methanol:Milli-Q (1:2:0.8 mL) solution for approximately 18 h. We centrifuged the solution at 1534 ×*g* for 2 min and transferred the supernatant into a glass tube, where we

separated the polar and non-polar phases using 1 mL each of dichloromethane and 0.9% NaCl saline solution and centrifuged at 1534 ×*g* for 2 min. The lower non-polar layer was transferred to a 2 mL vial and dried it under N_2 to a constant weight. We resuspended the total lipid extract in 1.5 mL dichloromethane and stored it at -80°C for subsequent fatty acid analysis.

We air-dried the lipid-extracted tissue overnight in a fume hood to remove residual solvents. After lipid extraction, we removed urea and TMAO using three rounds of sonication in deionised water, following Kim and Koch (2012). We then oven-dried samples at 60°C for 48 h, ground them to a fine powder for 3 min at 50 Hz using a TissueLyser LT (Qiagen) and prepared them for stable isotope analysis.

2.2 Fatty acid analysis

We analysed fatty acid profiles of 102 muscle (60 female; 42 male) and 39 plasma (26 female; 13 male) tissues following a modified Bligh and Dyer trans-methylation procedure (Bligh and Dyer, 1959; described in Meyer et al., 2017). An aliquot of the total lipid extract was trans-methylated using 3 mL of dichloromethane: methanol:hydrochloric acid (10:10:1 v/v/v) for 2 h at 80°C, then allowed to cool to room temperature before adding 1 mL of Milli-Q. We extracted the resulting fatty acid methyl esters (FAME) from the methylating solvent using three washes of 1.8 ml hexane: dichloromethane (4:1 v/v), centrifuged at 1534 ×*g* for 5 min, collected the upper solvent layer containing the FAMES in a 2 mL glass vial, dried under N_2 gas, and suspended it in 1 ml of dichloromethane. We identified and quantified individual fatty acids through gas chromatography (GC) and GC-mass spectrometry (GC-MS). We achieved peak separation using an Agilent Technologies 7890B GC (Palo Alto, California USA) with an Equity-1 fused silica capillary column (15 mm × 0.1 mm internal diameter and 0.1 mm film thickness), a flame ionisation detector, a splitless injector, and an Agilent Technologies 7683B Series auto-sampler. Samples were injected in splitless mode and carried by helium gas at an oven temperature of 120°C. The temperature was raised to 270°C at a rate of 10°C per min and then to 310°C at a rate of 5°C per min. We quantified fatty acid peaks using Agilent Technologies ChemStation software (Palo Alto, California, USA) and confirmed identities using a Finnigan Thermoquest DSQ GC-MS system. Fatty acids were converted from chromatogram peak area to percentage of total area.

2.3 Stable isotope analysis

We weighed 102 dried muscle (60 female; 42 male) and 38 plasma (25 female; 13 male) samples from white sharks (10–20 mg) into tin capsules using an automated microbalance described by Carvalho (2021) to ensure maximum accuracy. We analysed samples for $\delta^{13}C$ and $\delta^{15}N$ using an isotope ratio mass spectrometer (Thermo Delta V Plus) coupled to an elemental analyser (Thermo Fisher Flash EA) via an interface (Thermo Fisher Conflo IV). Isotopic ratios are expressed in delta (δ) values as the deviations from conventional standards in parts per thousand (‰) using the following formula: $\delta^{13}C$ or $\delta^{15}N = [(R_{sample}/R_{standard} - 1)] \times 1000$ (‰) where R_{sample} is the ratio of heavy to light isotope and $R_{standard}$ is the ratio of heavy to light isotope in the reference standard. We measured R_{sample} against internal working standards (glycine: $\delta^{13}C$ = -41.8, $\delta^{15}N$ = 2.0; glucose: $\delta^{13}C$ = -10.5; collagen: $\delta^{13}C$ = -21.5, $\delta^{15}N$ = 4.8), which were calibrated against international reference materials [(USGS64: $\delta^{13}C$ = -40.8, $\delta^{15}N$ = 1.8; USGS65: $\delta^{13}C$ = -20.3, $\delta^{15}N$ = 20.7; USGS64: $\delta^{13}C$ = -0.7, $\delta^{15}N$ = 40.8 (Schimmelmann et al., 2016)]. We reported $\delta^{13}C$ and $\delta^{15}N$ values relative to international reference materials V-PDB (Vienna-Pee Dee Belemnite) and atmospheric nitrogen (N_2) with a precision of 0.15 ‰ ($\delta^{13}C$) and 0.3 ‰ ($\delta^{15}N$).

2.4 Stable isotopes of prey samples

We collected muscle tissue samples from 18 potential white shark prey items to calculate prey contributions to white shark diet (Supplementary Table S1). Prey items were selected based on recent studies of immature white sharks in the same region (Grainger et al., 2020; Clark et al., 2023). Muscle samples were lipid-extracted using the above protocol, and urea was removed from elasmobranch prey samples using the outlined deionised water treatment. We could not obtain samples from cownose rays (*Rhinoptera neglecta*), so we used values from Raoult et al. (2019), which were not lipid extracted. To account for the effects of lipids on $\delta^{13}C$, these values were mathematically lipid-corrected before analyses using the below equation, described in Post et al. (2007).

$$\delta^{13}C_{normalised} = \delta^{13}C_{untreated} - 3.32 + 0.99 \times C:N$$

2.5 Statistical analysis

Of the 57 fatty acids detected, we used those with averages > 0.1% (14 muscle; 17 plasma; Table 1). We used PRIMER 7 + PERMANOVA (Permutational multivariate analysis of variance; Plymouth Routines in Multivariate Ecological Research, Anderson, 2017) to run multivariate statistical analyses, and R v4.3.1 (R Core Team, 2023) for linear and Bayesian mixing models.

PERMANOVA analysis (Type I, based on 9999 permutations) was used to investigate differences in white shark fatty acid profiles using season and sex as fixed factors, location as a random factor, and total length as a continuous covariate. PERMANOVA analyses were completed using a Euclidean distance matrix on normalised untransformed data. Pairwise comparisons were performed if the main PERMANOVA tests showed significant differences. Principal Coordinate Ordination analysis (PCO) was used to visualise the clustering of individuals and correlation between fatty acids.

If the PERMANOVA showed significant differences in fatty acid profiles, we further explored dietary patterns by fitting univariate linear models to a subset of fatty acids selected for their ecological relevance and high correlation on the PCO. We were unable to include location as a factor in the models because of the low sample

TABLE 1 Fatty acid relative composition (as percent of total fatty acid; mean ± SD) of white shark muscle and plasma collected in eastern Australia.

Fatty acid	Muscle (n = 102)	Plasma (n = 39)
14:0	1.85 ± 0.53	5.11 ± 1.62
15:0	0.46 ± 0.44	0.73 ± 0.36
16:0	36.75 ± 4.73	29.80 ± 1.95
16:1ω7	1.95 ± 0.62	2.49 ± 1.37
17:0	0.86 ± 0.21	1.46 ± 0.91
18:0	23.03 ± 4.81	16.31 ± 2.31
18:1ω9	9.90 ± 2.21	7.94 ± 2.30
18:2ω6	0.48 ± 0.17	1.01 ± 0.58
18:3ω2	0.00 ± 0.00	0.39 ± 0.28
20:0	0.00 ± 0.00	0.39 ± 0.19
20:1ω9	1.07 ± 0.50	0.63 ± 0.32
20:2ω6	0.17 ± 0.17	0.22 ± 0.24
20:4ω6 (ARA)	7.31 ± 2.40	9.72 ± 1.88
20:5ω3 (EPA)	1.18 ± 0.56	5.57 ± 1.22
22:0	0.00 ± 0.00	0.78 ± 0.43
22:6ω3 (DHA)	14.14 ± 7.3	15.95 ± 3.33
24:1ω9	0.55 ± 0.40	1.35 ± 0.51
Σ SFA	62.94 ± 2.42	51.78 ± 0.86
Σ MUFA	13.48 ± 0.83	12.40 ± 0.91
Σ PUFA	23.28 ± 3.04	35.92 ± 1.18

size across some locations (Supplementary Table S4). The effect of season (4 levels; fixed), sex (2 levels; fixed), and TL (continuous) on individual fatty acids (16:0, 18:0, 18:1ω9, ARA, EPA and DHA) was assessed by analysis of variance derived from each linear model. We used the same linear model to investigate changes in isotopic signatures in muscle and plasma:

$$y = X\beta + e$$

Where Y is the vector of fatty acid or stable isotope values observed on each shark and X is a matrix of indicator variables with columns representing season of capture, sex and TL. The vector β contains coefficients estimated by maximum likelihood. All models were tested for normality and homogeneity in the residuals (e). The models were also used to estimate the mean response to season with 95% confidence intervals.

We used Bayesian stable isotope mixing models of the muscle samples using the simmr package (Govan et al., 2023) to calculate the overall contributions of prey sources to the diet of white sharks. We excluded plasma stable isotope values from the analysis, as the C:N after lipid extraction was greater than the threshold (3.5), introducing potential bias to the mixing model results (Post et al., 2007; Hussey et al., 2012a). Before running the mixing model, we visually assessed the isotopic signatures of consumers, ensuring they were generally within the isospace of sampled prey (Supplementary Table S1). After the application of the trophic enrichment factor, the following prey did not fall within the isospace of the consumers and were removed from the model (Supplementary Figures S2, S3): cownose ray, pilchards (*Sardinops sagax*), tiger shark (*Galeocerdo cuvier*), and bronze whaler (*Carcharhinus obscurus*).

We used K-means clustering; assigning observations to the group with nearest centroid so that within group variance is minimised, to aggregate the remaining prey items (n = 13; Table 2) into three groups. This was recommended by Phillips et al. (2014), whereby model accuracy is greatest when the number of sources is equal to the number of tracers + 1. Stable isotope values of prey groups were adjusted for trophic enrichment using values for lipid-extracted sand tiger shark (*Carcharias taurus*) muscle from Hussey et al. (2011; $\delta^{13}C = 0.9 \pm 0.3‰$, $\delta^{15}N = 2.29 \pm 0.2‰$). We ran the mixing model with 3600 iterations over 4 MCMC chains and deemed model convergence satisfactory through a Gelman diagnostic value of 1.

We ran separate Bayesian mixing models on winter, spring, and summer to examine proportional prey contribution differences among seasons. Autumn was excluded from this analysis to avoid unreliable contributions, as only four samples were collected during

TABLE 2 Carbon and nitrogen isotope values (± SD) of grouped prey species used in the final Bayesian mixing model.

Prey group	Species	$\delta^{13}C$ (SD)	$\delta^{15}N$ (SD)
Low trophic level $\delta^{13}C$ enriched	*Trygonoptera testacea*	-17.4 (± 0.3)	12.0 (± 0.7)
	Urolophus sufflavus	-17.1 (± 0.4)	12.2 (± 0.1)
	Mugil cephalus	-16.7 (± 1.0)	12.0 (± 0.2)
Mid-trophic level $\delta^{13}C$ depleted	*Rhabdosargus sarba*	-17.5 (± 1.3)	12.9 (± 0.6)
	Scomber australasicus	-18.4 (± 0.2)	11.9 (± 0.3)
	Euthynnus affinis	-17.8 (± 0.4)	13.2 (± 0.3)
	Thunnus albacares	-17.5 (± 0.03)	12.7 (± 0.3)
	Trachurus novaezelandiae	-18.0 (± 0.1)	13.4 (± 0.2)
	Sepioteuthis australis	-17.7 (± 0.1)	13.1 (± 0.04)
High trophic level $\delta^{13}C$ enriched	*Carcharhinus obscurus*	-16.4 (± 0.3)	15.5 (± 0.7)
	Chrysophrys auratus	-16.6 (± 0.4)	14.5 (± 0.8)
	Thunnus obesus	-16.9 (± 0.2)	14.5 (± 0.1)
	Arripis trutta	-17.2 (± 0.1)	15.4 (± 0.2)

this season (Supplementary Table S4). Due to prey seasonality, two species, sea mullet (*Mugil cephalus*) and Australian salmon (*Arripis trutta*), were removed from the spring and summer models because their abundance is substantially reduced throughout these seasons (Lester et al., 2009; Hughes, 2012).

3 Results

3.1 Fatty acids

White shark muscle fatty acid profiles varied among seasons (PERMANOVA-pseudo-F = 4.34, p< 0.01) but were not influenced by sex or location (PERMANOVA- pseudo-F = 1.65, p > 0.05; Supplementary Table S5). Pairwise PERMANOVA revealed winter being different to both spring and summer, and autumn being different to both summer and spring (p< 0.05). Muscle samples collected in spring and summer had higher proportions of essential FA 20:4ω6 (ARA), 20:5ω3 (EPA), and 22:6ω3 (DHA) compared to samples collected in winter and autumn, which were dominated by saturated FAs 14:0, 16:0 and 18:0 (Figures 2, 3). Plasma fatty acid profiles, however, did not vary between season, sex, or location (Supplementary Table S5).

In muscle tissues, all six individual fatty acids were most affected by season, with autumn and winter having high 16:0, 18:0, and 18:1ω9, and low ARA, DHA, and EPA (Supplementary Tables S6, S7; Figure 3). Sex also affected muscle EPA and ARA (Supplementary Figure S8), while shark length affected muscle EPA and DHA (Supplementary Table S6; Supplementary Figure S9).

3.2 Stable isotopes

In white shark muscle tissue, $\delta^{13}C$ ranged from -14.6 to -17.5‰ (mean ± standard deviation: -16.2 ± 0.5‰), while $\delta^{15}N$ ranged from 14.6 to 16.4‰ (15.5 ± 0.3‰). Plasma $\delta^{13}C$ ranged from -14.3 to -17.8‰ (-15.3 ± 0.8‰) and $\delta^{15}N$ ranged from 12.4 to 14.9‰ (13.8 ± 0.5‰). Stable isotope values of muscle were influenced by seasons (Supplementary Table S10; Supplementary Figure S11), $\delta^{13}C$ was lowest in winter and $\delta^{15}N$ lowest in spring (Figure 4). Sex did not affect stable isotope values, but a weak but significant relationship existed in muscle and plasma $\delta^{15}N$ and total length (Supplementary Figures S12, S13).

3.3 Stable isotope mixing model and diet composition

Stable isotope values of white shark tissue were within the isoscape of potential prey sources (Figure 5; Supplementary Figure S3). Low trophic level $\delta^{13}C$ enriched prey were the primary resource used by immature white sharks and had an average contribution of 0.49 (± 0.04) to the annual diet of immature white sharks (Figure 6). High trophic level $\delta^{13}C$ enriched prey) contributed an average of 0.37 (± 0.05), while mid-trophic level $\delta^{13}C$ depleted prey contributed 0.14 to the diet of immature white sharks (± 0.01; Figure 6).

Seasonal mixing models showed substantial variation in prey proportions. Contributions from low trophic level $\delta^{13}C$ enriched prey) were highest in spring and summer, at 0.57 and 0.43 (± 0.04 and 0.02), respectively and decreased to 0.29 in winter (Figure 7). Mid-trophic level $\delta^{13}C$ depleted prey (contributed minimally during spring and summer at 0.07 and 0.14 (± 0.04 and 0.09), respectively

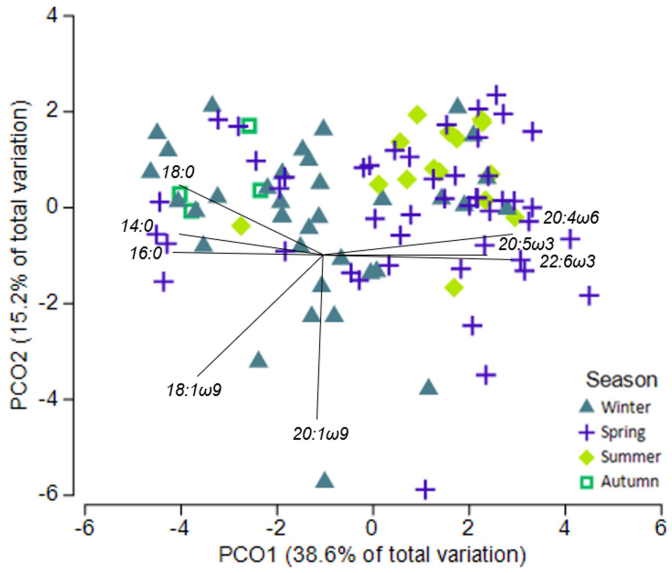

FIGURE 2
Principle coordinate ordination (PCO) of fatty acid profiles of white shark muscle and season. Vector overlay based on Pearson correlation with r > 0.7.

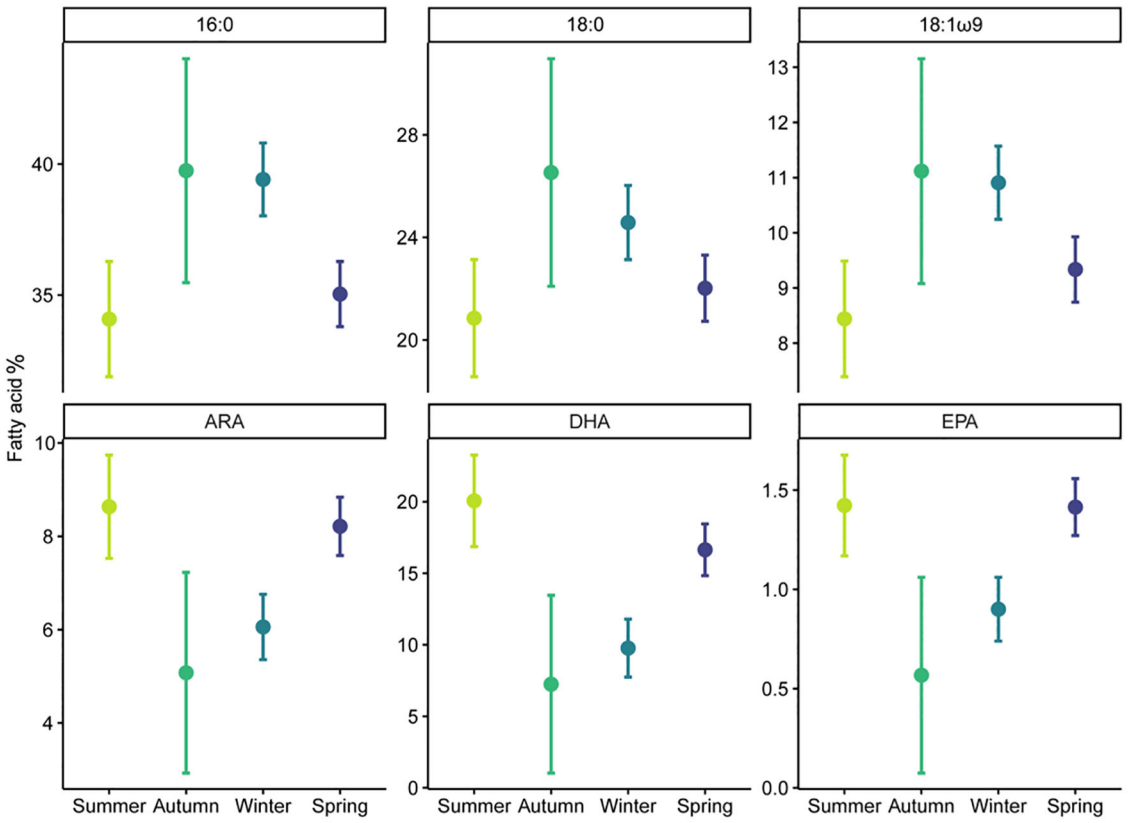

FIGURE 3
Seasonal variation (mean ± 95% CI) of fatty acid percentage for 16:0, 18:0, 18:1ω9, ARA (Arachidonic acid), DHA (Docosahexaenoic acid), and EPA (Eicosapentaenoic acid) in immature white shark muscle.

and increased to 0.40 (± 0.1) in winter (Figure 7). Proportions from high trophic level $\delta^{13}C$ enriched prey were lowest in winter, 0.31 (± 0.03), increased during spring to 0.36 (± 0.06), and were highest in summer at 0.43 (± 0.04; Figure 7).

4 Discussion

Short- and long-term dietary biomarkers provided a comprehensive overview of the diet and foraging habitat of immature white sharks on the east coast of Australia and revealed that they feed primarily on low-trophic prey in carbon-enriched coastal habitats. We found distinct seasonal differences in fatty acid signatures and diet composition from stable isotope mixing models. Specifically, muscle had lower proportions of essential polyunsaturated fatty acids, i.e., ARA, EPA, and DHA, during autumn and winter, suggesting that changes in prey availability linked to environmental factors, such as water temperature, influence white shark diet and foraging patterns. The increased contribution from inshore prey highlights the importance of these habitats as foraging grounds for immature white sharks, which is critical for their growth and development. Additionally, the seasonal diet shifts may influence these sharks' nutritional condition over a longer timescale as their energetic and nutritional requirements change throughout ontogeny.

4.1 Coastal habitat use and feeding

Fatty acid and stable isotope results suggest that white sharks use coastal habitats for foraging. Muscle and plasma ARA proportions were similar to previous studies in New South Wales and South Australia (Pethybridge et al., 2014; Meyer et al., 2017; Gallagher et al., 2019) and are comparable to other coastal sharks and benthic elasmobranchs (Davidson et al., 2011; de Sousa Rangel et al., 2021c; Zhang et al., 2023). ARA derives from benthic and coastal primary producers (Sardenne et al., 2017). Therefore, animals feeding in these areas would have elevated levels of this polyunsaturated fatty acid (Caraveo-Patiño et al., 2009; Hartwich et al., 2013) compared to those feeding in offshore habitats. Over a longer-temporal scale, $\delta^{13}C$ values support the fatty acid results of high coastal habitat use. However, the $\delta^{13}C$ values revealed high inter-individual variation, ranging between -14.6 to -17.5‰, which encompasses habitats dominated by coastal macrophytes (-14‰) and pelagic phytoplankton (-18‰; Hobson, 1999). The variation in $\delta^{13}C$ reported here indicate some individuals have a higher reliance on $\delta^{13}C$ depleted prey, similar to great hammerhead sharks (*Sphyrna mokarran*) that forage on coastal prey in the same region (Raoult et al., 2019).

Overall proportions of ARA and DHA indicate feeding on coastal prey. These fatty acids may reflect the contribution of fish

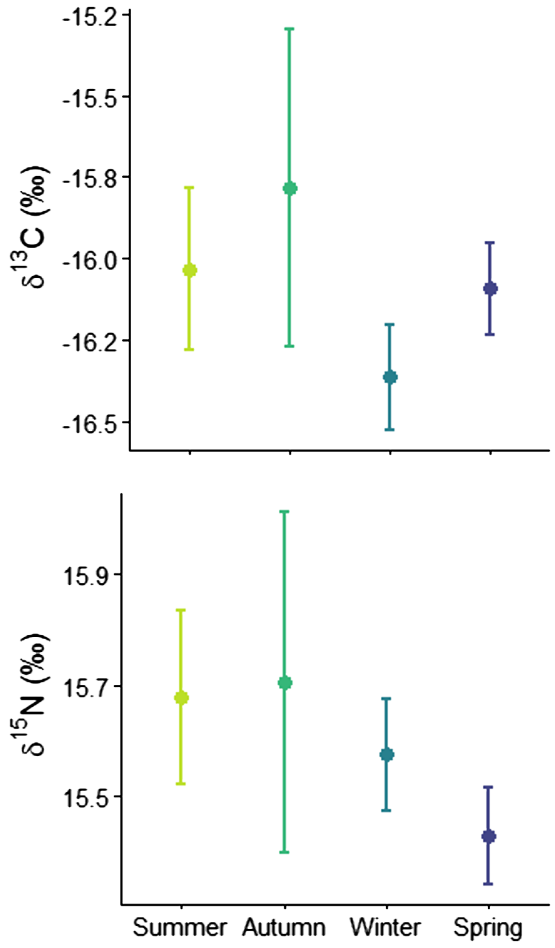

FIGURE 4
Seasonal variation (± 95% CI) of $\delta^{13}C$ and $\delta^{15}N$ (‰) of immature white shark muscle tissue.

and cephalopods to the diet of white sharks (Couturier et al., 2013; Pethybridge et al., 2014), with high ARA proportions representing benthic-feeding fishes and elasmobranchs (Couturier et al., 2013). This was further supported by the stable isotope Bayesian mixing model, showing white shark diet consisted primarily of low trophic level prey from coastal habitats with a substantial contribution from species at higher trophic levels These findings are similar to previous studies reporting that immature white sharks feed on various species from benthic and pelagic ecosystems (Hussey et al., 2012b; Tamburin et al., 2020). Previous stomach content and eDNA metabarcoding analyses showed that Australian salmon (*Arripis trutta*) and sea mullet (*Mugil cephalus*) are important prey for eastern Australian immature white sharks (Grainger et al., 2020; Clark et al., 2023). Benthic and benthopelagic elasmobranchs also contribute to the diet of immature white sharks in eastern Australia (Grainger et al., 2020) and in the North-Eastern Pacific Ocean (Tamburin et al., 2020) but were rarely detected in another study using eDNA metabarcoding of cloaca swabs (Clark et al., 2023). The differences in prey composition across these studies reflect the broad range of species consumed by white sharks, likely linked to seasonal variation in prey availability or broad-scale movements exhibited by most sharks, from Queensland to Victoria Spaet et al., (2020b). Additionally, the differences in $\delta^{13}C$ and $\delta^{15}N$ values seen here, indicate there are substantial dietary variations among individuals which may not be reflected in the contributions from the mixing model.

4.2 Seasonal plasticity in diet

Stable isotope mixing models from muscle samples showed seasonal shifts in diet, with substantial changes from low and higher trophic level $\delta^{13}C$ enriched prey in spring and summer to mid-

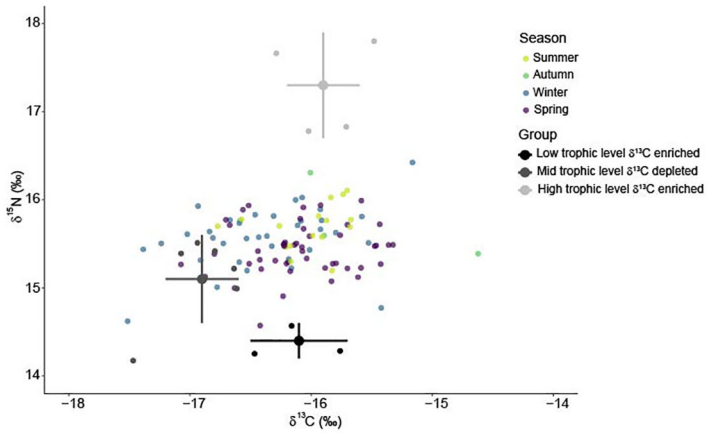

FIGURE 5
Biplot of white shark muscle $\delta^{13}C$ and $\delta^{15}N$ values (coloured by season) compared to the mean (± standard deviation) of three prey groups used in the Bayesian mixing model. Low trophic level $\delta^{13}C$ enriched (■), mid-trophic level $\delta^{13}C$ depleted (■), high trophic level $\delta^{13}C$ enriched (□).Prey values are corrected for trophic enrichment based on Hussey et al. (2010): + 2.3‰ $\delta^{15}N$ values and + 0.9‰ $\delta^{13}C$ values.

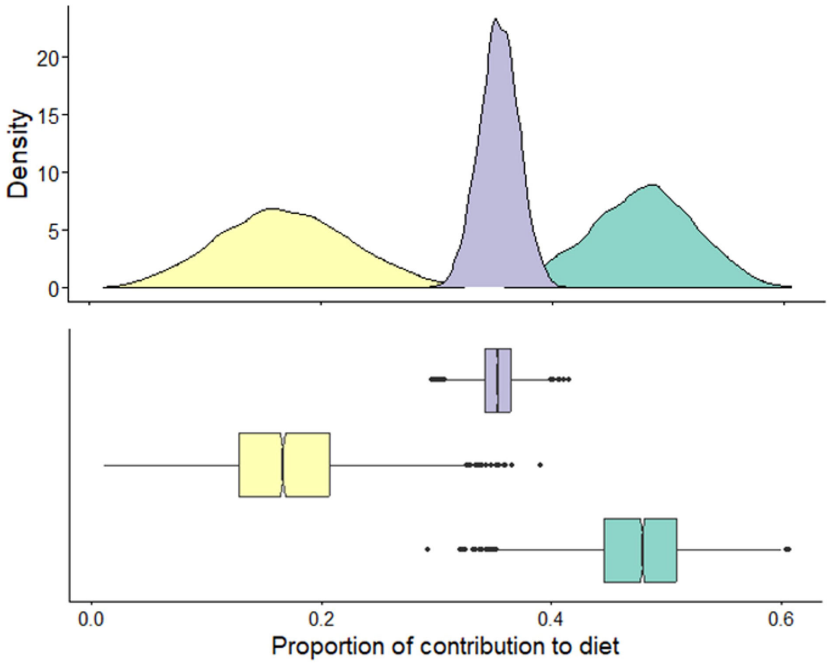

FIGURE 6
The proportional contribution of prey groups estimated from Bayesian mixing models for white shark muscle collected in New South Wales, Australia, between 2020-2021 (low trophic level $\delta^{13}C$ enriched; mid-trophic level $\delta^{13}C$ depleted; high trophic level $\delta^{13}C$ enriched).

trophic level $\delta^{13}C$ depleted prey in winter. The fatty acid results further support this dietary shift. Proportions of DHA increased substantially during the spring and summer months and are similar to those found in benthic elasmobranchs (Dunstan et al., 1988; El Kebir et al., 2007) and bigeye tuna (*Thunnus obesus*; Peng et al., 2013). Fatty acid 18:1ω9 is a strong indicator of mesopelagic fish, such as yellowfin tuna (*Thunnus albacares*; Sardenne et al., 2016) and cephalopods (Phillips et al., 2001; Meyer et al., 2019), suggesting higher consumption of these prey during autumn and winter when this fatty acid increases. Seasonal variations in environmental conditions can impact the distribution and abundance of prey (Poloczanska et al., 2007; Last et al., 2011). The strengthening EAC during late spring causes persistent upwelling over shelf waters, subsequently increasing productivity (Roughan and Middleton, 2002). This coincides with white shark presence and capture in the region, which peaks during winter and spring, when water temperatures are below 21°C (Spaet et al., 2020a; Lipscombe et al., 2023). Consequently, the seasonal changes detected in our study are likely linked to the changes in prey availability associated with water temperature and season. The East Australian Current strongly influences water temperatures (Malcolm et al., 2011) and prey distribution on the east coast of Australia (Booth et al., 2007). Thus, these factors also influence shark occurrence and capture (Spaet et al., 2020a; Lipscombe et al., 2023), and habitat use by potential prey species (Gillanders et al., 2001; Zischke et al., 2012). Fatty acids are known to be influenced by sea surface temperature (Dalsgaard et al., 2003; Meyer et al., 2019); therefore, by combining these with stable isotopes, we have greater confidence in the inferences of diet. Future studies should focus on how changes in prey distribution and availability in response to the strengthening East Australian Current may impact the trophic ecology of immature white sharks.

4.3 Changes in nutritional condition among seasons

Seasonal variations in diet have the potential to impact the nutritional condition of white sharks. The higher proportions of saturated fatty acids and lower proportions of essential dietary fatty acids in autumn and winter suggest that prey consumed in these months is of lower nutritional quality or certain prey species are less abundant. Saturated fatty acids are ubiquitous in all animals and, combined with monounsaturated fatty acids, are the primary fatty acids catabolised for energy (Tocher, 2003). In contrast, polyunsaturated fatty acids are conserved for critical biological processes (Tocher, 2010). Lipid metabolism is relatively understudied in elasmobranchs, yet unlike teleost fishes, lipid storage occurs in the large liver (Zammit and Newsholme, 1979). The proportions of saturated fatty acids in muscle may suggest a metabolic adjustment to compensate for the lack of high-quality prey. Conversely, substantial increases in the proportions of the essential fatty acids, particularly DHA, in spring and summer imply a shift in diet to prey that supports cellular functions and physiological processes, such as growth, during these periods. Although the seasonal change in diet is evident, links to nutritional condition and the implications of poor nutrition in sharks are speculative and should be examined in more detail in

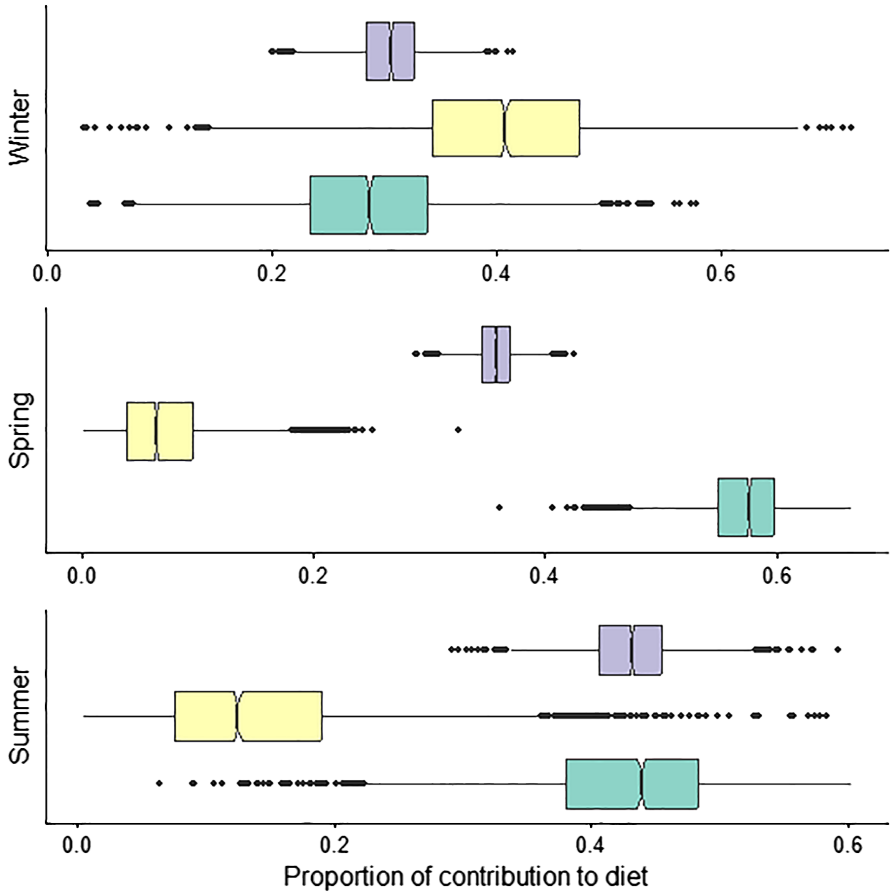

FIGURE 7
Seasonal Bayesian mixing models of white shark diet using muscle (autumn was excluded from these analyses due to small sample size) collected in New South Wales, Australia, between 2020-2021 (low trophic level $\delta^{13}C$ enriched; mid-trophic level $\delta^{13}C$ depleted: high trophic level $\delta^{13}C$ enriched). Sea mullet and Australian salmon were excluded from the spring and summer model.

future studies. Additionally, assessing responses and metabolic adaptations to dynamic coastal ecosystems may be invaluable for forecasting distribution changes associated with anthropogenic impacts.

4.4 Ontogenetic changes in diet

Ontogenetic diet shifts are well documented in white sharks from the Pacific (Estrada et al., 2006; Kim et al., 2012b). Due to limitations in sample availability, few (n = 8) samples were collected from sharks > 3 m; therefore, only a small increase in $\delta^{15}N$ was detected with shark length. Furthermore, there was high variability among individuals, with a smaller shark (2.5 m) having the highest $\delta^{15}N$ value and larger sharks exhibiting values below 15.5‰, lower than those recorded for immature sharks in Mexico and the north-east Pacific (Carlisle et al., 2012; Tamburin et al., 2020).

An animal's energy and nutritional requirements typically increase with size (Gallagher et al., 2014). Although the relationships between fatty acid proportions and length are yet to be described in white sharks, the negative relationships seen here are vastly different from those of other shark species (de Sousa Rangel et al., 2021a; de Sousa Rangel et al., 2021c). We expected fatty acid proportions to increase with size as larger and higher quality prey were consumed. However, we observed decreasing proportions of DHA and EPA in muscle. Increases in DHA with total length were found in the plasma of tiger sharks (*Galeocerdo cuvier*) in the Atlantic (de Sousa Rangel et al., 2021a), where larger mature sharks exhibited higher values compared to immature sharks, which was linked to nutritional adjustments in preparation for reproduction. Similar results have been observed for mature male blacktip sharks in EPA, DHA and ARA (de Sousa Rangel et al., 2021b). Based on these results, it is plausible that our results reflect the immature life stage of white sharks, where energetic investments for reproduction are not yet required. More extensive sampling of mature white sharks across a broader spatial scale may assist in clarifying the relationships between total length and specific fatty acids when sharks are closer to maturity.

4.5 Experimental considerations

Consideration must be given to the limitations of using bulk tissue stable isotopes in trophic ecology studies. Specifically, mixing models are sensitive to trophic enrichment factors and isotopic

routing, which can lead to inaccurate results (Phillips et al., Bond and Diamond, 2011). Species- and tissue-specific trophic enrichment factors are limited for large sharks, as controlled feeding experiments are impractical. Currently, no trophic enrichment factor is available for white sharks, and as a large-bodied predator occupying a high trophic level, there are several physiological (e.g., growth rate, isotope routing) and environmental (e.g., temperature) factors that may impact the discrimination of stable isotopes (Caut et al., 2009; Hussey et al., 2010; Phillips et al., 2014). Subsequently, the value provided by Hussey et al. (2010) from multiple species of sharks was deemed the best alternative. Isotopic routing remains unclear in shark tissues, yet it is well documented to cause significant issues when interpreting mixing models in teleosts, resulting in over or underestimating the contribution of sources to diet (Kelly and Martínez del Rio, 2010). We also acknowledge that while we aimed to include a range of prey items of white sharks, $\delta^{13}C$ values indicate many of these sharks are feeding on coastal prey with values around -15‰, that were not included in the mixing model. There may also be another prey group between the low trophic and high trophic level $\delta^{13}C$ enriched groups that these sharks are feeding on that we have been unable to sample in this study. One of the many limitations of mixing models is their inability to detect missing prey or differentiate if consumers values are the average of two separate prey groups with distinct isotopic signatures (Phillips et al., 2014; Stock et al., 2018). Additionally, the more prey included in the mixing model, the less precise the estimates of contribution (Phillips et al., 2014). Furthermore, interpretation of stable isotopes requires caution, as spatial and temporal variation of isotopes exists in marine environments and are influenced by environmental and anthropogenic factors (Pethybridge et al., 2018; Matich et al., 2021).

5 Conclusion

We examined the diet and habitat use of immature white sharks on the east coast of Australia using a combination of fatty acid and stable isotope analyses. White sharks are highly dependent on coastal resources with high contributions from low trophic level coastal prey, corroborating previous studies of white shark diet in this region. There was evidence of a seasonal shift in diet, which may be associated with changes in prey availability with varying water temperatures. These seasonal changes may influence the nutritional condition of white sharks and negatively affect physiological processes that support health and growth. This study expands on the recent dietary studies of white sharks, providing a holistic understanding of a species that is typically characterised as a generalist predator. Yet, the seasonal variations detected here reveal that their diet is more complex than previously described. Further studies investigating how ocean warming may impact prey distribution and white shark foraging would prove valuable, in addition to changes in the biochemistry and metabolism of white sharks and what implications this may have on the nutritional condition of these sharks.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by NSW DPI and Southern Cross University Animal Care and Ethics Committee (ACEC 07/08: ARA 21/034). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

RL: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. LM: Conceptualization, Formal analysis, Writing – review & editing. PB: Conceptualization, Writing – review & editing. SM: Formal analysis, Writing – review & editing. CH: Conceptualization, Formal analysis, Writing – review & editing. AS: Conceptualization, Supervision, Writing – review & editing. PB: Conceptualization, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2024.1359785/full#supplementary-material

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